Influx of Calcium into Rabbit Myocardium in Relation to Its Ionic Environment*

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Abstract. In the first 150 seconds after contracture of rabbit myocardium has been induced by shifting from perfusion with zero [Ca\(^{2+}\)] and low [K\(^+\)] to solutions with normal levels of those cations, there is a large influx of Ca\(^{2+}\) as measured both by isotopic tracer flux and by total tissue [Ca]. Tracer studies indicate that the influx is 90 per cent complete in 90 seconds. Contracture due to substitution of either Li\(^+\) or K\(^+\) for Na\(^+\) in perfusion fluids is also associated with an increased influx, but of lesser magnitude. The latter types of contracture are reversible while the former is not. It seems probable that the irreversible contracture is induced by the large Ca\(^{2+}\) influx.

Previous studies\(^1\) have shown that the net influx of Ca\(^{2+}\) into heart muscle after a period of exposure to zero [Ca\(^{2+}\)] perfusion fluid is highly dependent upon the [K\(^+\)] during the period of Ca\(^{2+}\) lack. If the [K\(^+\)] during such a period is 3 mM, the restoration to normal of perfusate [Ca\(^{2+}\)] results in a sustained, strong, and irreversible contracture, along with a very large net gain in tissue [Ca]. If the [K\(^+\)] concentration during Ca\(^{2+}\) lack is 5 mM, there is no significant change in tissue [Ca] when the latter cation is restored to the perfusion system. This paper will report the results of studies on the time parameters of Ca\(^{2+}\) exchange under these and some other conditions.

Methods. Rabbit hearts, removed under nembutal anesthesia, were perfused at constant rates with oxygenated isosmotic solutions of specified compositions at 37°C by the Langendorff method.\(^2\) The standard salt solution employed contained NaCl 142.95, Na\(_2\)HPO\(_4\) 2.05, KCl 4.85, KH\(_2\)PO\(_4\) 0.15, CaCl\(_2\) 1.8, glucose 5 mM. Other perfusate compositions are noted in the table of results. Muscle [Ca] was determined in ashed tissue by the method of Klass.\(^3\) The extracellular Ca\(^{2+}\) was subtracted, on the basis of sucrose-space measurements and assay of perfusate [Ca\(^{2+}\)], on the assumption that there would be equilibrium as to [Ca\(^{2+}\)] in the perfusate effluent and the extracellular, extracellular fluid. \(^{45}\)Ca was employed as a tracer for calcium movement by switching the perfusate source to a reservoir containing the isotope at the time of beginning the Ca\(^{2+}\) uptake period. After this period the perfusion was instantaneously shifted to solutions of identical composition except for the Ca\(^{2+}\) isotope. Nonradioactive perfusion was continued for exactly 180 sec in order to eliminate the majority of the high activity perfusate. The perfusate effluent was collected at short intervals and it was assumed that the extracellular space was in equilibrium with the perfusate during the final collection period. The 180 sec washout was employed because Ca\(^{2+}\) entrance into muscle cells is slow and large errors could be introduced into \(^{45}\)Ca values for the intracellular compartments by small errors in sucrose-space measurements. According to previous studies on dog papillary muscle by Langer\(^4\) and others, the half time for the

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escape from the extracellular compartment is about 72 sec while that for the most rapidly exchanging tissue compartment is 360 sec. The results reported here are therefore those for net isotopic influx remaining in the tissue after 3 min of washout. Uptake was calculated on the basis of the assumption that there was no back diffusion of isotope. This assumption is not justified for determination of absolute unidirectional flux rates and no such rates are considered. The differences, depending upon prior treatment rather than the absolute values, are the significant results of the study.

In the main series of experiments to be reported, the *45Ca loading time was 150 sec. In another series the loading times were extended at intervals, up to 1 hr.

**Results and Discussion.** The findings are expressed in μmole Ca\(^{2+}\) net influx/g dry ventricular tissue during the period of loading. As noted above, these values really represent the labeled Ca\(^{2+}\) that remains in the tissue after a 3-mm washout. Table 1 shows that in a rhythmically contracting heart perfused with

<table>
<thead>
<tr>
<th>Perfusion solutions and times (mM)</th>
<th>No. of experiments</th>
<th>State of contraction with final perfusate</th>
<th>Ca(^{2+}) influx in 150 sec (μmole/g dry ventricular muscle ± SE)</th>
<th>Ventricular muscle Ca (μmole/g dry wt. ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na 145, Ca*1.8, K 5 (150 sec)</td>
<td>6</td>
<td>Rhythmic contraction</td>
<td>0.94 ± 0.007</td>
<td>18.9 ± 0.4</td>
</tr>
<tr>
<td>Na 145, Ca 0, K 5 (12 min)</td>
<td>6</td>
<td>Rhythmic contraction</td>
<td>0.72 ± 0.012</td>
<td>15.4 ± 0.3</td>
</tr>
<tr>
<td>Na 147, Ca 0, K 3 (12 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>→ Na 145, Ca*1.8, K 5 (150 sec)</td>
<td>6</td>
<td>Reversible contracture</td>
<td>5.73 ± 0.10</td>
<td>23.2 ± 0.5</td>
</tr>
<tr>
<td>Na 145, Ca 1.8, K 5 (150 sec)</td>
<td>6</td>
<td>Reversible contracture</td>
<td>2.24 ± 0.15</td>
<td>20.5 ± 0.6</td>
</tr>
<tr>
<td>Na 145, Ca 1.8, K 5 (150 sec)</td>
<td>5</td>
<td>Reversible contracture</td>
<td>1.95 ± 0.15</td>
<td>20.4 ± 0.8</td>
</tr>
<tr>
<td>→ Na 0, Ca*1.8, Li 150 (150 sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Isotopically labeled.
† Labeled calcium remaining in tissue after 3-min washout.

the standard salt solution, 5% of the total tissue Ca\(^{2+}\) exchanged with that in the perfusate in 150 sec. In the second series of experiments shown, the zero [Ca\(^{2+}\)] perfusion brought about mechanical arrest, and the reintroduction of Ca\(^{2+}\) during isotopic loading resulted in a return of contracture. The influx and/or exchange of Ca\(^{2+}\) was calculated to be 4.7% of that present at the end. In the third series, the [K+] in the perfusate was 3 mM in the period of zero [Ca\(^{2+}\)] and restoration of the Ca\(^{2+}\) resulted in contracture. The Ca\(^{2+}\) influx and/or exchange was 24.8% that of the final tissue [Ca] or 37.2% of that which was presumably in the muscle at the end of the zero perfusate [Ca\(^{2+}\)] period, based upon the value 15.4 μmole/g dry tissue found in the second series in the table. The gross net Ca uptake was presumably 7.8 μmole/g dry tissue. This value is somewhat higher than the labeled Ca\(^{2+}\) influx (5.73 μmole/g dry tissue) and is probably to be explained by the fact that back diffusion has been ignored and especially by the fact that during the 3-min nonradioactive washout period the back diffusion of isotope from the most rapidly exchanging compartment re-
sulted in loss of proportionately more $^{40}$Ca during washout than would have occurred if the isotope were randomly distributed through all compartments in the muscle. That is to say that exchange diffusion between the rapidly exchanging compartment and the vascular compartment during washout results in an underestimate of influx during loading at all times. Consequently the failure to find complete agreement between the increase in total tissue [Ca] and the calculated influx is not surprising. The points that are significant, however, are that the influx rate as calculated is at least six times greater than normal ($P \ll 0.01$) and that total tissue [Ca] is very much elevated ($P < 0.01$).

In the cases of substitutions of K$^+$ or Li$^+$ for Na$^+$, there was the expected contracture and there was an approximate doubling of the Ca$^{2+}$ influx over normal ($P < 0.01$). The increase in tissue [Ca] of about 5% is of borderline statistical significance. In all cases in which contracture occurred there were highly significant increases in the rate of influx and/or exchange of Ca$^{2+}$. It appears that the contracture may be a result of this phenomenon. Niedergerke showed that in frog ventricle the substitution of K$^+$ or Li$^+$ for Na$^+$ resulted in a large increase in Ca$^{2+}$ influx. In the complete absence of Na$^+$ the increase was 30 times more than normal.

The second series of experiments was performed in order to compare the time course of tissue [Ca] in the irreversible contracture dependent upon low [K$^+$] during zero [Ca$^{2+}$] perfusion followed by exposure to normal cation concentration perfusion, with other conditions. In Figure 1 are presented the data on

![Figure 1](image-url)

uptake over time of loading and total tissue Ca content for three conditions: perfusion with standard salt solution during the contracture produced as described above, rhythmic contracture during perfusion with standard salt solution, and perfusion with standard salt solution after a period of arrest produced by zero [Ca$^{2+}$] and 5 mM [K$^+$] in the perfusate. Each point represents the mean of three or four experiments.

It may be noted that the hearts perfused only with standard salt solution gained total [Ca] slowly over the hour, as did those that had been depleted by
zero \([\text{Ca}^{2+}]\), 5 mM \([\text{K}^+]\) in prior treatment. The latter had not reached complete restoration within the hour of perfusion with standard salt solution. The total tissue \([\text{Ca}]\) in the contracture series showed a prompt rise within the first 3 min. The isotopically measured uptake reached 90% of its maximum value within 1.5 min. The decline in tissue \([\text{Ca}]\) after 20 min means that in the state of contracture there was some net loss of \(\text{Ca}^{2+}\). There was also a loss, rather than a gain, in \(^{44}\text{Ca}\) in the tissue during the period from 10 to 30 min of perfusion. The loss in total Ca was about 2 \(\mu\text{mole/g dry tissue}\). The loss in labeled \(\text{Ca}^{2+}\) was about 1 \(\mu\text{mole/g dry tissue}\). Thus there was isotopic exchange occurring still, as would be expected.

It is not possible to use the data presented to estimate absolute rates of influx or exchange because, as noted under methodology, there was a washout with nonradioactive perfusates before the hearts were frozen for analysis. Consequently isotope exchange from the rapidly exchanging tissue compartments with the vascular compartment occurred. Therefore, conclusions concerning the uptake studies must be limited to comparisons between the several experimental procedures. It seems certain that there is an extremely rapid uptake of \(\text{Ca}^{2+}\) within the first 90 sec of the time contracture begins in the situation reported. In another study\(^1\) we have found that other large chemical changes with respect to phosphoryl creatine and ATP occur within this period. It also seems certain that in this type of irreversible contracture the tissue \([\text{Ca}]\) remains elevated well above normal throughout at least an hour, during which the tension declines, but not to resting values.

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